Safety Data Sheet

Dichloro-methotrexate

Division of Safety
National Institutes
of Health

WARNING!

THIS COMPOUND IS MODERATELY TOXIC AND CARCINOGENIC. IT IS READILY ABSORBED THROUGH THE SKIN AND RESPIRATORY AND INTESTINAL TRACTS. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND COLD WATER. AVOID WASHING WITH SOLVENTS. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, DRINK PLENTY OF WATER OR MILK. INDUCE VOMITING. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. SEE CASTEGNARO ET AL. (1985) FOR DETAILS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

Introductory note: There is little specific information on the chemical, physical, and biological properties of dichloromethotrexate in the literature. Much of what is presented here is based on properties of the parent compound methotrexate. Where specific information exists and is not evident from the text it is indicated by an asterisk.

Issued: 1/87

Prepared by the Environmental Control and Research Program

A. Background

Dichloromethotrexate (DCM) is a yellow-orangeA crystalline compound stable in pure form and in neutral solution but unstable in excess acid and alkali or under ultraviolet light. It is about one-tenth as toxic as methotrexate; there are no data concerning mutagenicity teratogenicity, and embryotoxicity, and its carcinogenicity is slight. DCM is an antineoplastic inhibitor of folic acid metabolism, probably acting, as does methotrexate, by strongly (but reversibly) inhibiting dihydrofolate reductase (DHFR).

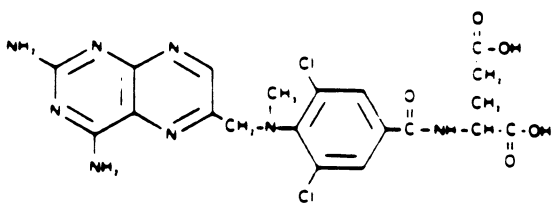
The properties of DCM have been reviewed (Ensminger, 1984).

B. Chemical and Physical Data

1. Chemical Abstract No.: 528-74-5.

2. Synonyms: 3',5' Dichloroamethopterin; 4-amino-10-methyl-3',5'-dichloropteroylglutamic acid; glutamic acid, N-[3',5'-dichloro-4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl;B NSC 29630, NCI-CO 4875.

3. Chemical structure and molecular weight:



$C_{20}H_{20}Cl_2N_8O_5$; 523.35

4. Density: No data.

*5. Absorption spectroscopy: Spectral data (λ_{max}, ε) are: in 0.1 N NaOH: 258, 25,600; 370, 7,600; in 0.1 N HCl: 240, 23,100; 330 12,100 (Angier and Curran, 1959). Fluorescent data are: λ_{exc} = 420, λ_{em} = 480 nm (Pavlik et al., 1983).

AThis color is assumed on the basis of ultraviolet absorption data which are similar to those of methotrexate.

BChemical Abstract name, used for listing in 5th Decennial Index and subsequently.

6. Optical rotation: No data.

7. Volatility: No data; may be regarded as essentially non-volatile.

8. Solubility: No data but probably low in water, higher in dilu alkali, hydroxide, or carbonate. Stated to be "ten times more lipid soluble than methotrexate" (Weinstein and McCullough, 1975).*

9. Description: Yellow-orange (see footnote A above) platelets from 50% aqueous ethanol.

10. Boiling point, melting point: No data.

11. Stability: No data; probably similar to methotrexate which has high heat stability but is photolyzed in aerated solution under ultraviolet light.

12. Chemical reactivity: No data but may be expected to be subject to hydrolysis, oxidation, and ring substitutions. Metabolic reactions are discussed in F, below.

13. Flash point: No data.

14. Autoignition temperature: No data.

15. Explosive limits in air: No data.

Fire, Explosion, and Reactivity Hazard Data

1. DCM is likely to be inactivated under conditions of fire. Fire fighting personnel should wear protective clothing and face masks.

2. Flammability is likely to be low.

3. Conditions contributing to instability are acid, alkali, elevated temperatures, and prolonged exposure to ultraviolet light.

4. Hazardous decomposition products under conditions of fire are likely to include nitrogen oxides and hydrochloric acid.

Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially

carcinogenic chemicals are used in NIH laboratories. The NIH Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving DCM.

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation: Validated methods have been reported (Castegnaro et al., 1985).

2. Decontamination: Turn off equipment that could be affected by DCM or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Consult Castegnaro et al. (1985) for details concerning decontamination of surfaces, glassware, and animal cages.

3. Disposal: It may be possible to decontaminate waste streams containing DCM before disposal. For details, see Castegnaro et al. (1985). No waste streams containing DCM shall be disposed of in sinks or general refuse. Surplus DCM or chemical waste streams contaminated with DCM shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing DCM shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing DCM shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with DCM shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing DCM shall be handled in accordance with the NIH radioactive waste disposal system.

4. Storage: Store solid DCM and its solutions in dark-colored, tightly closed containers, preferably under refrigeration. Avoid exposure to light and moisture. Store working quantities of DCM and its solutions in an explosion-safe refrigerator in the work area.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data but methods of preparation of biological samples should be the same as those used for the determination of methotrexate (reviewed by Stout et al., 1985). The main methods are either protein precipitation with perchloric acid (Watson et al., 1978), acetonitrile (Chen and Chiou, 1981), or boiling (Krackower and Kamen, 1983) followed by extraction; or by precolumn deproteinization (Breithaupt et al., 1982; So et al., 1985) when HPLC is the method of analysis.

2. Analysis: In recent years high-pressure liquid chromatography (HPLC) has been the almost exclusive method of choice because of sensitivity and specificity (separation of DCM from methotrexate and their metabolites). Specific applications to DCM have been described by Tony et al. (1980). However, it is quite likely that appropriate variations of this method, developed for methotrexate and reviewed in the Safety Data Sheet for this compound, can also be applied.

Biological Effects (Animal and Human)

1. Absorption: DCM is absorbed and produces biological effects after parenteral (intravenous, intraperitoneal) injection and probably by ingestion. In vitro experiments have shown that DCM is absorbed through human normal and psoriatic skin and is a better DHFR inhibitor by this route than is methotrexate (Weinstein and McCullough, 1975). However, systemic effects of DCM when administered by this route have not been demonstrated.

2. Distribution and pharmacokinetics: Most of the information of DCM distribution is based on experiments with Cl^{36}-labeled material and thus does not take account of possible metabolic transformation. In the mouse, one hour after parenteral administration radioactivity is detected in all tissues but predominantly in liver, small intestine, and kidney. Within 48 hours, two-thirds of the activity is found in the feces, most of the remainder in the urine, with about 1% retention in the liver. Similar results were obtained in the rat, rabbit, and dog (Oliverio and Davidson, 1962). No pharmacokinetic studies of DCM appear to have been carried out.

3. Metabolism and excretion: The major metabolic product of DCM in the animal body is the 7-hydroxy derivative, but the extent of this oxidation is highly species dependent. In the dog, there is no metabolism and all DCM is excreted unchanged (Loo and Adamson, 1965); in the mouse and rat, metabolism is quite low, while in the rabbit and guinea pig it is extensive. This is in line with in vitro studies of the activity of liver aldehyde oxidase in several species: high specific activity in guinea pig, intermediate in rabbit, and low in mouse and rat

(Johns et al., 1966; Bertino et al., 1967). This represents a detoxication mechanism since 7-OH DCM is approximately one fourth as toxic as DCM, and would explain the relative resistance of the mouse and rat to DCM (and methotrexate). Excretion of DCM and/or its metabolite is via bile and urine, again varying with species; in man about equal amounts are excreted by each route (Davidson and Oliverio, 1965).

There is no published evidence whether the anabolic formation of polyglutamates, so prominent with methotrexate, occurs with DCM also.

4. Toxic effects: The acute LD50 of intravenous DCM is 1,000 mg/kg and the "lethal dose" in monkeys is 400 mg/kg (Hacker et al., 1978). DCM would, therefore, appear to be considerably less toxic than methotrexate. Toxic effects have been found in liver, bone marrow, gastrointestinal tract, and lymphoid tissue, but there is little evidence of the nephrotoxicity usually associated with methotrexate (Ensminger, 1984). The chief mechanism of toxic action is the stoichiometric, competitive inhibition of dihydrofolate reductase (DHFR), resulting in depletion of 1-carbon carrying tetrahydrofolate cofactors which are important in the synthesis of thymidilic and inosinic acid for DNA and RNA production.

5. Carcinogenic effects: Weisburger (1977), in a comprehensive survey of many chemotherapeutic agents, reports a 1.5-2 fold increase in tumor incidence over controls when DCM was administered. This is the only report on the subject.

6. Mutagenic and teratogenic effects: No data.

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. Skin should not be rinsed with organic solvents. Since DCM is readily absorbed through the skin, avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with sodium bicarbonate solution, followed by copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.

2. Ingestion: Drink plenty of water or milk. Induce vomiting. Refer for gastric lavage.

3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.

4. Refer to physician.

References

Angier, R.B., and W.V. Curran. 1959. The halogenation of 4-amino-4-deoxy-N^{10}-methylpteroic acid (Methotrexate) and other pteroylglutamic acid derivatives. J Am Chem Soc 81:2814-2818.

Bertino, J.R., B.L. Hillcoat, and D.G. Johns. 1967. Folate antagonists: Some biochemical and pharmacological considerations. Fed Proc 26:893-89.

Breithaupt, H., E. Künzlein, and G. Goebel. 1982. Rapid high-pressure chromatographic determination of methotrexate and its metabolites 7-hydroxymethotrexate and 2,4-diamino-N^{10}-methylpteroic acid in biological fluids. Anal Biochem 121:103-113.

Castegnaro, M., J. Adams, M.A. Armour, J. Barek, J. Benvenuto, C. Confalonieri, U. Goff, S. Ludeman, D. Reed, E.B. Sansone, and G. Telling. 1985. Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Antineoplastic Agents. IARC Scientific Publications No. 73. World Health Organzation, Geneva, Switzerland.

Chen, M.-L., and W.L. Chiou. 1981. Sensitive and rapid high-performance liquid chromatographic method for the simultaneous determination of methotrexate and its metabolites in plasma, saliva and urine. J Chromatog 226:125-134.

Davidson, J.D., and V.T. Oliverio. 1965. The physiologic disposition of dichloromethotrexate-Cl^{36} in man. Clin Pharmacol Ther 6:321-327.

Ensminger, W.D. 1984. Clinical pharmacology of folate analogs; in: Sirotnak, F.M., J.S. Burchall, W.D. Ensminger, and J.A. Montgomery (eds)., Folate Antagonists as Therapeutic Agents. 2:133-163. Academic Press, NY.

Hacker, M.P., C.B. Hong, L.C.K. Wong, C.C. Lee, and D.A. Cooney. 1978. A toxicologic evaluation of dichloromethotrexate (NSC-29630) and/or leuovorin (NSC-3590) in dogs and monkeys. Report PB-29120; Chem Abstr, 91:83278z.

Johns, D.G., A.T. Iannotti, A.C. Sartorelli, and J.R. Bertino. 1966. The relative toxicities of methotrexate and aminopterin. Biochem Pharmacol 15:555-561.

Krackower, G.R., and B.A. Kamen. 1983. In situ methotrexate polyglutamate formation in rat tissues. J Pharm Exp Ther 227:633-638.

Loo, T.L., and R.H. Adamson. 1965. The metabolite of 3',5'-dichloro-4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid (dichloromethotrexate). J Med Chem 8:513-515.

Oliverio, V.T., and J.D. Davidson. 1962. The physiological disposition of dichloromethotrexate-Cl^{36} in animals. J Pharm Exp Ther 137:76-83.

Pavlik, E.J., D.E. Kenady, J.R. van Nagell, K. Keaton, M.B. Hanson, E.S. Donaldson, W.D. Griffen, and R.C. Flanigan. 1983. Properties of anticancer agents relevant to in vitro determination of human tumor cell sensitivity. Cancer Chemother Pharmacol 11:8-15.

So, N., D.P. Chandra, and I.S. Alexander. 1985. Determination of serum methotrexate and 7-hydroxymethotrexate concentrations. Method evaluation showing advantages of high-performance liquid chromatography. J Chromatog 337:81-90.

Stout, M., Y. Ravindranath, and R. Kauffman. 1985. High performance liquid chromatographic assay for methotrexate utilizing a cold acetonitrile purification and separation of plasma and cerebrospinal fluid. J Chromatog 342:424-430.

Tony, W.P., J.L. Wisnicki, J. Horton, and D.B. Ludlum. 1980. A direct analysis of methotrexate, dichloromethotrexate, and thei 7-hydroxy metabolites in plasma by high-pressure liquid chromatography. Clin Chim Acta 107:67-72.

Watson, E., J.L. Cohen, and K.K. Chan. 1978. High-pressure liquid chromatographic determination of methotrexate and its major metabolite, 7-hydroxymethotrexate, in human plasma. Cancer Chemother Rep 62:381-387.

Weinstein, G.D., and J.L. McCullough. 1975. Effects of methotrexate esters on normal and psoriatic skin. Arch Dermatol 111:471-475.

Weisburger, E.K. 1977. Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. Cancer 40:1935-1949.